

Effect of sex and haplotype on plasma tryptase levels in healthy adults

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Background: The total level of α -tryptase and β -tryptase in serum or plasma is used as a clinical indicator of the mast cell burden.

Objective: The effect of the tryptase haplotype and of sex on the total tryptase level of healthy individuals was determined.

Methods: A novel hot-stop PCR technique was used to determine the tryptase genotype, and a standard fluoroenzyme immunoassay was used to measure total plasma tryptase levels in 106 healthy subjects. Mx modeling and the QTL association routine of Mendel 5.0 were used to analyze the data.

Results: Tryptase haplotypes exhibit a 1 ($\beta\alpha/\beta\alpha$):2 ($\beta\beta/\beta\alpha$):1 ($\beta\beta/\beta\beta$) distribution, monomorphic for β at 1 position and allelic for β and α at the other position. The $\beta\alpha$ haplotype has a frequency of 0.49. The $\beta\alpha$ haplotype increases total tryptase levels by 0.5 ng/mL from the overall mean, whereas female sex increases the level by 0.2 ng/mL from the mean.

Conclusion: The tryptase haplotype and sex each have a statistically significant effect on the total plasma tryptase level of healthy subjects. (J Allergy Clin Immunol 2004;114: 48-51.)

Key words: Tryptase, mast cells

The genes for α -tryptase and β -tryptase are preferentially expressed in human mast cells.¹ Levels in serum and plasma of the pro forms of α -tryptase and β -tryptase reflect the number of mast cells in the body, whereas levels of the mature form of β -tryptase, normally undetectable (<1 ng/mL), are transiently elevated during systemic anaphylaxis in proportion with the degree of hypotension.² The commercially available total tryptase

immunoassay (UniCAP, Pharmacia Diagnostics, Uppsala, Sweden) measures pro and mature forms of α -tryptase and β -tryptase.³ Under nonanaphylactic conditions, only α -protryptase and β -protryptase are likely present in serum and plasma. Under such conditions, total tryptase levels range from 1 to 15 ng/mL in healthy subjects, whereas patients with systemic mastocytosis typically have levels >20 ng/mL.⁴ Total tryptase levels are also elevated in some patients with various refractory anemias and myelodysplastic or myeloproliferative diseases, particularly subsets of acute myelogenous leukemia.^{5,6} The haploid genotype for tryptase is either $\beta\alpha$ or $\beta\beta$, ie, there are 2 tryptase genes on human chromosome 16p13.3, a monomorphic copy of β -tryptase and an allelic copy of either α -tryptase or β -tryptase. Consequently, diploid individuals may have a $\beta\alpha/\beta\alpha$, $\beta\beta/\beta\alpha$, or $\beta\beta/\beta\beta$ genotype. Both haplotypes are common. In fact, approximately 25% of subjects are α -tryptase-deficient.^{3,7,8}

The effect of haplotype on total tryptase levels has been partially assessed in 109 healthy subjects, and no significant difference was found between the median levels of those with (5.2 ng/mL) and those without (4.7 ng/mL) an α -tryptase gene.³ However, the technique used to assess tryptase genes in that study did not distinguish between the haplotypes of subjects with $\alpha:\beta$ gene ratios of 2:2 and 1:3, ie, those with 1 or both haplotypes bearing α -tryptase and β -tryptase genes, potentially obscuring a gene-dosage effect. The current study used a hot-start PCR technique to define more precisely the tryptase haplotypes of these same subjects and concluded that there was a modest but statistically significant effect of both genotype and sex on the total plasma tryptase level in healthy subjects.

METHODS

PBS, MES, HEPES, TRIS, sodium azide, EDTA, BSA, agarose, and $MgCl_2$ (Sigma Chemical Co, St Louis, Mo); dNTP mix (Boehringer Mannheim, Indianapolis, Ind); Genomic DNA purification kit, RNase-free DNase, T4 polynucleotide kinase, *Bst*EII, and *Eco*RV (Promega, Madison, Wis); ProbeQuant G-50 Micro column (Amersham, Piscataway, NJ); AmpliTaq (Perkin-Elmer Corp, Foster City, Calif); and polyacrylamide gels (Invitrogen, Carlsbad, Calif) were obtained. The total tryptase assay was performed on the UniCAP platform as recommended.³ Primers were prepared and

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sequencing was performed by the Nucleic Acid Core facility at Virginia Commonwealth University.

Analysis of the relationship between the presence and absence of the gene for α -tryptase and plasma tryptase levels

Genomic DNA had been purified by using a salting-out method from peripheral blood leukocytes derived from EDTA-anticoagulated venous blood and was available from 106 (56 male subjects, 50 female subjects) of the original 109 subjects (PureGene; Gentra, Minneapolis, Minn). These healthy individuals ranged in age from 21 to 52 years (mean $\pm$ SD, 27.5 ± 6.1) and provided informed consent as approved by the human studies Internal Review Board of Virginia Commonwealth University. Study subjects were chiefly medical and graduate students and were not selected or characterized on the basis of atopy. On the basis of medication histories, 8 female and 3 male subjects reported treatment for rhinitis; 2 female and 3 male subjects reported treatment for asthma. For genomic DNA, hot-stop PCR was conducted with 50 ng genomic DNA in a total volume of 50 μ L containing $1\times$ reaction buffer with 200 μ mol/L dNTP and 5 pmol/L of each primer (sense, 5'-GGAGCAGCACCTCTACTACC-3'; antisense, 5'-GGCGCCAAGGTGGTATTTTGC-3') as described.⁹ The sense and antisense primers were located in exons 4 and 5, respectively, and amplified 432 bp (α -tryptase) and 443 bp (β -tryptase) regions from genomic DNA. The optimal $MgCl_2$ concentration for PCR was 0.5 mol/L. Hot-start reactions (95°C for 5 minutes) were initiated with 1 U Taq DNA polymerase followed by 35 cycles of amplification in a volume of 50 μ L. Each cycle consisted of 1 minute at 94°C, 1 minute at 62°C, and 1 minute at 72°C. To eliminate α/β tryptase heteroduplexes from the analysis of PCR products, hot-stop PCR was performed as described.¹⁰ The antisense primer was labeled with [γ -³²P] ATP by using T4 polynucleotide kinase, and the labeled DNA was then separated from unincorporated labeled nucleotides by chromatography on a ProbeQuant G-50 Micro column. With ³²P-labeled antisense primer added before the final cycle of amplification, only homoduplexes incorporate radioactivity. Any heteroduplexes formed during previous cycles would be unlabeled. The corresponding PCR products derived from genomic DNA were digested with *EcoRV* and *BstEII* restriction enzymes to digest α -tryptase and β -tryptase, respectively. *EcoRV* digests of the α -tryptase PCR products yielded 341-bp and 91-bp fragments. *BstEII* digests of the β -tryptase PCR products yielded 298-bp and 145-bp fragments. The digested PCR products were subjected to electrophoresis in 12% polyacrylamide gels followed by β -scanning and analysis with Quantity One software (Bio-Rad Laboratories, Hercules, Calif). Total plasma tryptase levels from the 106 healthy subjects had been determined previously.³

Statistical analyses

The genetic modeling describing effects of sex and tryptase gene haplotype was performed with Mx (www.vipbg.vcu.edu)¹¹ and also with the QTL association routine of Mendel 5.0 (www.genetics.ucla.edu/software).¹²

RESULTS

Tryptase genotypes

The experimental $\beta:\alpha$ tryptase gene ratios of the 106 study subjects, determined by using the hot-stop PCR technique, clearly distributed into 4:0, 3:1, or 2:2 ratios. Only β -tryptase DNA was detected in the 4:0 genotype group of 28 subjects; this defined the $\beta\beta/\beta\beta$ combination of haplotypes. The $\beta:\alpha$ gene ratios in the 3:1 genotype group of 47 subjects ranged from 2.89 to 3.52 and yielded

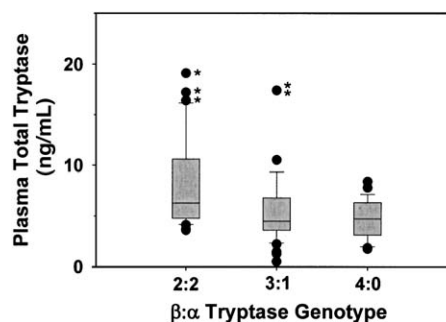


FIG 1. Effect of tryptase genotype on plasma total tryptase levels. Total tryptase levels are grouped according to genotype and shown as box and whisker plots with median values (central hatch marks), 25th/75th percentiles (box ends), 10th/90th percentiles (whiskers) and outliers (closed circles). *Five outliers eliminated for the Mendel quantitative trait association modeling.

a mean $\pm$ SD of 3.08 ± 0.13 ; this defined the $\beta\beta/\beta\alpha$ combination of haplotypes. The $\beta:\alpha$ ratios in the 2:2 genotype group of 31 subjects ranged from 0.90 to 1.24 and yielded a mean $\pm$ SD of 1.02 ± 0.08 , defining the $\beta\alpha/\beta\alpha$ haplotype combination. This experimental 28:47:31 distribution of haplotype combinations fit the predicted $p^2:2pq:q^2$ distribution for Hardy-Weinberg equilibrium ($\chi^2 = 1.3$; 1 df; $2p = 0.25$). The frequency of the haplotype bearing both α -tryptase and β -tryptase was 0.49.

PCR products derived from several of the genomic DNA samples were not completely digested with *BstEII*. Sequencing of the indigestible portion showed that it was identical to β -tryptase except for a nt421A>G polymorphism in exon 4, which resulted in a 141Thr $\rightarrow$ Ala variation, as reported previously.⁹ This T141A polymorphism disrupted the *BstEII* cleavage site and was detected in 8 of the 4:0 (29%) and 7 of the 3:1 $\beta:\alpha$ (15%) genotype groups, but in none of the 2:2 $\beta:\alpha$ genotype group. A previous analysis showed the β -tryptase polymorphism to be present in DNA from 0 of 6 subjects with a 2:2 $\beta:\alpha$ genotype and from 2 of 7 subjects with a 3:1 $\beta:\alpha$ genotype.⁹ The T141A polymorphism was in linkage disequilibrium with the $\beta\beta$ haplotype (using GOLD software [www.well.ox.ac.uk/asthma/GOLD], $\chi^2 = 11.33$; $P = .00076$; $\Delta^2 = 0.072$; $D' = 1.00$).¹³ Thus, the pattern was consistent with a T141A polymorphism present in about 10% of the allelic β -tryptase gene copies and not on the monomorphic gene copy found in both haplotypes.

Tryptase genotype association with plasma total tryptase levels

Plasma total tryptase levels for each tryptase genotype are shown in Fig 1, where median values (25th, 75th percentiles) are 6.3 (4.8, 10.4), 4.5 (3.6, 6.7), and 4.7 (3.3, 6.3) ng/mL. A trend of higher total tryptase levels in the 2:2 $\beta:\alpha$ genotype group was apparent. The presence of the β -tryptase polymorphism noted had no significant effect on total tryptase levels within either the 4:0 or 3:1 $\beta:\alpha$ genotype groups. Total tryptase levels higher than 8.5 ng/mL were observed only in those with at least 1 α -tryptase

gene. Also, total tryptase levels had previously been noted to be slightly higher in female subjects than male subjects.³ Statistical modeling was used to correct for sex effects and to assess genetic influences. Mendel software was used to examine quantitative trait associations and provided results that described deviations upward or downward from a grand mean tryptase concentration of 5.1 ng/mL. Five outliers (Fig 1, *asterisks*) were omitted to reduce skewness. Female subjects were responsible for each of these outliers. The best fitting model included sex and tryptase haplotype. Although the subject distribution was skewed toward a younger population, models including age effects did not fit as well as those omitting age. The independent effect of the tryptase $\beta\alpha$ haplotype was to increase the tryptase mean by 0.5 ng/mL ($P = .002$), whereas the tryptase $\beta\beta$ haplotype was constrained to reduce it equally. The sex effect was to increase tryptase from the mean by 0.2 ng/mL for female subjects and reduce it by 0.2 ng/mL for male subjects. Thus, Mendel estimated female means for the respective haplotypic combinations $\beta\alpha/\beta\alpha$, $\beta\alpha/\beta\beta$, and $\beta\beta/\beta\beta$ were 6.8, 5.7, and 4.7 ng/mL, respectively; male values were 0.4 ng/mL lower for each haplotypic combination. Mx models omitting sex effects or genetic effects did not fit as well as those including sex and genetic influence. Mx results were described by deviations from the grand mean tryptase level of 5.9 ng/mL for the $\beta\alpha/\beta\alpha$ haplotypic combination. The effect of male sex was to reduce tryptase by 0.25 ng/mL from the mean, and female sex increased by an identical level. The additive genetic effect of each $\beta\beta$ haplotype was to reduce tryptase by 1.0 ng/mL, and the dominance variance effect was -0.9 ng/mL, ie, heterozygote values were mean minus 1.0 ng/mL for each $\beta\beta$ haplotype and minus an additional 0.9 ng/mL for the heterozygous state. In this context, female tryptase means for haplotypic combinations $\beta\alpha/\beta\alpha$, $\beta\alpha/\beta\beta$, and $\beta\beta/\beta\beta$ were predicted at 6.2, 4.2, and 4.1 ng/mL, respectively; male values were 0.5 ng/mL lower for each category. Sex and tryptase haplotype accounted for 11.6% of all tryptase variation in Mx modeling. These modeling tools therefore not only confirmed the previously described sex influence (plasma total tryptase levels higher in female subjects than male subjects) but also showed a modest tryptase haplotype influence on plasma total tryptase levels ($\beta\alpha$ haplotype higher than $\beta\beta$). Whether atopy affected the total tryptase level or was influenced by the tryptase genotype was not addressed in the current study. However, a previous analysis of total serum tryptase levels in 62 subjects with active allergic rhinitis and 19 healthy controls did not detect a difference.¹⁴

DISCUSSION

In summary, the $\beta\alpha$ tryptase genotype was determined in 106 healthy subjects by a novel hot-stop technique, which eliminated confounding heteroduplexes from the analysis. This technique yielded genotype frequencies that

were consistent with the $\beta\beta$ and $\beta\alpha$ haplotypes previously predicted (1:2:1 $\beta\beta/\beta\beta:\beta\beta/\beta\alpha:\beta\alpha/\beta\alpha$) and reported.^{8,15,16} The percentage of subjects who were α -tryptase-deficient (26%) was comparable with the 29% ($n = 274$),⁸ 20% ($n = 60$),⁷ and 26% ($n = 109$)³ values previously reported. Importantly, small but statistically significant effects of tryptase haplotype and sex on the plasma total tryptase level were noted, with the $\beta\alpha$ haplotype and female sex giving higher values. The effect of these observations on the clinical use of total tryptase levels in serum or plasma remains to be fully assessed but should be considered when interpreting test results. The mechanisms for these effects have not been explored. However, it is enticing to speculate that a higher portion of the product of the α -tryptase than β -tryptase gene might be spontaneously secreted. This is because processing of α -protryptase, if it occurs at all, is likely to be much less efficient than that of β -protryptase,¹⁷ and it is primarily α -protryptase and β -protryptase that are spontaneously secreted by mast cells.³ In contrast, the effect of sex could be on the total body burden of mast cells, the production and processing of tryptase by mast cells, or the metabolism of spontaneously secreted tryptase.

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